

What is claimed is:

1. A method for determining polynucleotide expression comprising:
 - a) providing at least one target polynucleotide, said polynucleotide having a 3' end and a 5' end;
 - b) providing a first oligonucleotide primer, wherein a portion of said first primer
5 is capable of hybridizing to said target polynucleotide;
 - c) obtaining a first strand cDNA by reverse transcription of said target polynucleotide, said first strand cDNA having a 5' end and a 3' end, wherein said 5' end of said first strand cDNA contains a sequence corresponding to said first oligonucleotide primer and said 3' end of said first strand cDNA comprises at least one nucleotide that
10 extends beyond the 5' end of said target polynucleotide to provide a single-stranded extension;
 - d) providing a second oligonucleotide primer, wherein at least a portion of said second oligonucleotide primer is capable of hybridizing to said single-stranded extension;
 - e) extending said first strand cDNA using said second oligonucleotide primer as a
15 template to produce an extended first strand cDNA containing said first oligonucleotide primer and a region complementary to said second oligonucleotide primer;
 - f) amplifying said extended first strand cDNA in the presence of at least one detectable label to produce amplified cDNA such that said amplified cDNA contains said at least one detectable label;
 - 20 g) digesting said amplified cDNA to produce a digested cDNA;
 - g) hybridizing said digested cDNA to a capture probe coupled to a solid particle under stringent conditions wherein said capture probe is specific for said target polynucleotide and said particle identifies said capture probe; and
 - h) determining if said digested cDNA has hybridized to said capture probe
25 thereby identifying said target polynucleotide.
2. The method of claim 1, wherein said particle comprises a fluorescent particle.

3. The method of claim 2, wherein said fluorescent particle comprises a fluorescent microbead or microsphere.
4. The method of claim 2, wherein said fluorescent particle comprises a plurality of groups of fluorescent particles, the particles of each group having a unique fluorescent signature and comprising a capture probe specific for a single target polynucleotide.
5. The method of claim 4, wherein said fluorescent particles comprise microbeads or microspheres.
6. The method of claim 5, wherein determining if said digested cDNA has hybridized to said capture probe thereby identifying said target polynucleotide is accomplished by flow cytometry.
7. The method of claim 6, wherein multiple capture probes hybridize to the same target polynucleotide at different locations on said target polynucleotide.
8. The method of claim 7, wherein said first oligonucleotide primer comprises the sequence $n_y(t)_xvn$, where x is an integer between 4 and 50 and y is an integer between 10 and 50.
9. The method of claim 8, wherein said second oligonucleotide primer comprises the sequence $n_y(g)_x$, where y is an integer between 10 and 50 and x is an integer between 1 and 6.
10. The method of claim 9 wherein said first oligonucleotide primer and said second oligonucleotide primer contain a restriction site.
11. A method for diagnosing a disease, condition, disorder or predisposition in a test subject comprising, determining polynucleotide expression in a test subject by the method of claim 1; determining polynucleotide expression in a reference subject known

- to have said disease, condition, disorder, or predisposition by the method of claim 1; and
- 5 comparing polynucleotide expression in said test subject to polynucleotide expression in said reference subject.

12. A method for determining the physiological or developmental state of a cell or tissue comprising, determining polynucleotide expression in a test cell or tissue by the method of claim 1; determining polynucleotide expression in a reference cell or tissue of a known physiological or developmental state by the method of claim 1; and comparing
- 5 polynucleotide expression in said test cell or tissue to polynucleotide expression in said reference cell or tissue.

13. A method for detecting a single nucleotide polymorphism comprising:

- a) providing at least one primer pair, said primer pair containing a reverse primer and a forward primer comprising a 3' end specific for an allele of a single nucleotide polymorphism of interest and a hybridization tag that identifies the primer, said
- 5 hybridization tag not complementary to the sequence containing said single nucleotide polymorphism of interest;

b) combining said at least one primer pair with a sample containing single-stranded polynucleotides under stringent conditions which allow hybridization of said primers to complementary sequences in said single-stranded polynucleotides;

- 10 c) extending hybridized primers by primer extension to produce an extension product wherein said extension product comprising said hybridization tag and a detectable label;

- d) hybridizing said extension products by said hybridization tag or the complement thereof under stringent conditions to a capture probe wherein said capture
- 15 probe is coupled to a particle, said particle identifying said capture probe;

e) detecting the hybridization of said extension product to said capture probe by the presence of said detectable label; and

f) determining the identity of said single nucleotide polymorphism based on the identity of said particle.

14. The method of claim 13, wherein said reverse primer comprises said detectable label.

15. The method of claim 14, wherein said reverse primer pair is a universal reverse primer.

16. The method of claim 13, wherein c) is repeated at least once.

17. The method of claim 13, wherein said at least one primer pair comprises a plurality of primer pairs specific for a plurality of single nucleotide polymorphisms.

18. The method of claim 13, wherein said detection is by flow cytometry.

19. A method for diagnosing a disease, condition, disorder or predisposition in a subject comprising, obtaining a biological sample containing at least one polynucleotide from said subject and analyzing said at least one polynucleotide to detect the presence or absence of a single nucleotide polymorphism by the method of claim 13, wherein said single nucleotide polymorphism is associated with a disease, condition, disorder or predisposition.

20. A method for detecting a single nucleotide polymorphism comprising:

a) providing at least one oligonucleotide primer comprising a hybridization tag that identifies said primer, said primer having a 3' end specific for a single nucleotide polymorphism of interest;

b) combining said at least one primer with a sample containing single-stranded polynucleotides under stringent conditions which allow hybridization of said primer to complementary sequences in said single-stranded polynucleotides;

c) extending hybridized primers by primer extension to produce an extension product, said extension product comprising said hybridization tag and a detectable label;

- 10 d) hybridizing said extension product by said hybridization tag under stringent conditions to a capture probe, said capture probe couple to a particle that identifies said capture probe;
- e) detecting the hybridization of said extension product to said capture probe using said detectable label; and
- 15 f) determining the identity of said single nucleotide polymorphism based on the identity of said particle.

21. The method of claim 20, wherein said at least one primer comprises a plurality of primers each specific for a different single nucleotide polymorphism.

22. The method of claim 20, wherein said at least one primer comprises a group of at least 2 primers, each primer in said group having a 3' end specific for a different allele of a single nucleotide polymorphism of interest.

23. The method of claim 22 further comprising a plurality of said primer groups, each primer group specific for a different single nucleotide polymorphism of interest.

24. The method of claim 20, wherein the 3' end of said primer is immediately adjacent to location of the single nucleotide polymorphism of interest.

25. The method of claim 24, wherein said primer extension is a single base primer extension.

26. The method of claim 25, wherein said single base extension is achieved by using only a single type of nucleoside triphosphate.

27. The method of claim 25, wherein said single base extension is achieved by using at least one-chain terminating nucleoside triphosphate.

28. The method of claim 27, wherein said chain-terminating nucleotide-triphosphate is a dideoxynucleoside triphosphate.

29. The method of claim 25, wherein said single base extension is achieved by using a plurality of chain-terminating nucleoside triphosphates, each comprising a unique label.

30. The method of claim 29, wherein said chain-terminating nucleotide triphosphates are dideoxynucleoside triphosphates.

31. A method for diagnosing a disease, condition, disorder or predisposition in a subject comprising, obtaining a biological sample containing at least one polynucleotide from said subject and analyzing said at least one polynucleotide to detect the presence or absence of a single nucleotide polymorphism by the method of claim 20, wherein said
5 single nucleotide polymorphism is associated with a disease, condition, disorder or predisposition.

32. A method for selecting hybridization tags comprising identifying non-coding sequences of between about 10 to about 30 nucleotides long, wherein said sequences lack hairpin structures and duplex-forming abilities; identifying those sequences having a GC content of between about 40% to about 50% and a T_m that varies by no more than 2°C ;
5 and selecting such sequences as hybridization tags.

33. A hybridization tag produced by the method of claim 32.

34. A universal hybridization tag comprising a nucleotide sequence selected from
10 the group consisting of SEQ ID NOS 3, 4, 5, 6, 9, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25, 26, 28, 29, 30, 31, 32, 36, 38, 40, 41, 42, 43, and 45.

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